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# Effects of Hofmeister ions on solubility and swelling on gelatin hydrogels

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# Effects of Hofmeister ions on Solubility and Swelling of Gelatin-based Hydrogels

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**Honors Research Project**

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**Executive Summary**

Purpose

Hydrogels have the potential to be used for a wide range of applications. One of the most prominent applications are in tissue engineering, which include drug delivery and wound healing. Hydrogels are known for their hydrophilic, or water attractive, properties, but they are often brittle and weak. There is a need for stronger, more durable, hydrogels for some of these biomedical applications. The most attractive hydrogels for this need are gelatin-based hydrogels due their biocompatibility, biodegradability, and low cost. These gels can be deswelled and toughened by soaking in different aqueous solutions containing kosmotropic Hofmeister's ions. These ions strengthen the gelatin-based hydrogels efficiently at a low cost.

Results

To determine if hydrogels could be strengthened by using salt solutions containing Hofmeister's ions, three experiments were conducted. All studies used the same protein-based gelatin and six different salt solutions containing Hofmeister's ions. The six salts used throughout were sodium chloride, sodium sulfate, sodium carbonate, sodium thiocyanate, calcium chloride and ammonium chloride.

The first experiment tested the solubility of gelatin in various concentrations of salt solutions at three temperatures (4 °C, room temperature, and 37 °C). This data acted as a guideline for the experiments to follow. It was important to determine at which concentration of solution the gelatin was soluble at because that demonstrated a breakdown of the hydrogel network. The results showed that gelatin was easily soluble in sodium thiocyanate and calcium chloride at all temperatures. The rest of the salt solutions showed varying amounts of solubility and helped identify concentrations best suited for the following studies.

The second part of this project was a swelling study. The solubility study data was vital as swelling tests should not be completed for any solution in which the gelatin would just dissolve. Since gelatin was soluble in all molarities tested for sodium thiocyanate and calcium chloride tested, it was determined to not proceed with those salts. 10 wt% hydrogels were made and weighed, then soaked in salt solutions of various molarities. The hydrogels were dried and weighed after 2 hours and again at 24 hours to obtain a mass ratio. Mass ratios greater than 1

indicated swelling, while mass ratios less than 1 indicated the sample deswelled or shrunk. Gelatin soaked in DI water, 2 M sodium chloride, and 1 M – 3 M ammonium chloride all swelled. Swelling was present after 2 hours, but the hydrogels continued to swell more over time, giving larger mass ratios after 24 hours. 3 M ammonium chloride experienced some disintegration, in which the hydrogel was starting to fall apart after soaking for 2 hours. For sodium sulfate and sodium carbonate, the swelling/deswelling depended on the molarity of solution. Both salt solutions showed swelling at 0.5 M and 0.75 M, but they deswelled in both 1 M and 2 M solutions.

The final experiment performed in this study was aimed to determine whether gelatin was released into the solution post-swelling tests or not. This was done by creating a calibration curve and then testing the absorbance of solutions after gelatin was soaked for 24 hours. The calibration curve determined the concentration (wt %) present in the solution. All solutions tested for the various salts indicated very close to zero gelatin present, except ammonium chloride. The 4 M $NH_4Cl$ solution showed the most gel released (average of 0.23 wt%) due to the gelatin dissolving into the solution as seen during soaking.

## Conclusions from Results

Based on all the results obtained from the project, it is inferred that the gelatin-based hydrogels is strengthen by using salt solution containing Hofmeister's ions. This is promising as it is a cost-effective way to easily obtain these tougher hydrogels that may be used in many applications. It was observed that gelatin will deswell, and become stronger, when soaked in 1 M or 2 M sodium sulfate or sodium carbonate solutions. All other salts tested in this project, sodium chloride, sodium thiocyanate, calcium chloride and ammonium chloride will cause the hydrogels to swell when soaked and become weaker.

## Recommendations

There are many directions in which future work could be completed using the ideas from this project. It was shown that hydrogels can be deswelled and toughened by soaking in certain salt solutions containing Hofmeister's ions. First, the tougher hydrogels were obtained using sodium sulfate and sodium carbonate. Higher molarity solutions should be studied to determine if there is room for making the gels even stronger or what would happen if the solution was too strong. Additionally, swelling tests were concluded after 24 hours. It would be interesting to see what happens when the hydrogels are soaked longer. There is a threshold for when significant swelling/deswelling stops, but that is unknown currently. Also, knowing now which Hofmeister's ions resulted in stronger hydrogels, others can be analyzed. Ions of similar strength should be tested to determine if they show similar results. A suggestion on how to better analyze solubility data is to create tertiary phase diagrams. This would allow the easy visualization of how temperature, gel concentration, and salt solution concentration impacts solubility. This could make the selection of molarities for salt solution simpler. It could also save time in only testing solutions that should show promising results.

## Broader Implications

This project has given me the opportunity to grow my research and time management skills. I was given a lot of responsibility in planning and running my own experiments, analyzing data and drawing conclusions from that data, and learning new techniques not taught in a classroom. I was able to practice my time management and learn how to organize my experiments to be efficient. I had to also learn how to recover and communicate with my research advisor when experiments did not work out or I came across an issue. I have grown a lot technically from this experience. The results from my project also have the potential to be useful in the biomedical field. Since gelatin-based hydrogels are inexpensive, biodegradable, and biocompatible, once they are tougher than the average hydrogel, they have potential to be used in many more applications.

**Introduction**

Hydrogels are formed through polymer crosslinks and are known to absorb and hold large amounts of water, making them weak and brittle [1]. Although hydrogels are used in many applications, including tissue engineering, wound healing and drug delivery, there is a need for mechanically strong hydrogels for these and other applications. One of the simplest approaches to obtain tougher hydrogels is by forming gelatin-based gels which are treated with different aqueous solutions containing kosmotropic Hofmeister's ions. The kosmotropic ions enhance salting out of proteins (i.e., taking water away from protein-based gels). Prior research has shown that these ions strengthen gelatin hydrogels and increase their mechanical properties [10].

In Dr. Newby's Research Lab for Surface Modification and Materials, there have been previous studies conducted to toughen hydrogels using Hofmeister's ions, especially when using gelatin hydrogels. Gelatin-based hydrogels were the focus of the studies as these hydrogels would be used for biomedical applications. A biocompatible and biodegradable hydrogel, especially an in-expensive protein-based hydrogel, is needed when using it for the human body. Earlier studies in Dr. Newby's lab have focused on following gelation of gelatin by using 0.1 M salt solutions as solvents to form gels. Because of the low salt concentration, there were no issues in gel formation and solubility. There is a need for further studies using salt solutions of higher concentrations. Hydrogels have the possibility to be stronger when formed in higher salt concentration solutions, making it possible for them to be used in applications that they are not used for today. The gelatin hydrogels could also improve their toughness by soaking the gels in solutions containing kosmotropic Hofmeister ions with sufficiently high enough concentration.

The objectives of this project were to examine the solubility of gelatin in salt solutions, determine the swelling effects Hofmeister ions have on gelatin hydrogels, and find the optimal conditions to form the strongest hydrogels. Through solubility studies, trends were made showing how temperature, salt solution concentration, and gelatin concentration impact solubility. This data was used further for the swelling studies. Through a variety of swelling tests, an optimal combination of temperature and salt solution containing a Hofmeister ion was found to strengthen the hydrogels through swelling/deswelling.

**Background**

Gelatin-based hydrogels

A hydrogel is a 3-dimensional network of hydrophilic polymer chains and networks. Hydrophilic, or "water loving", means that these polymer chains are attracted to water. This attraction to water results in some hydrogels having the ability to absorb as much as 800 times its original weight in water [2]. The ability to absorb so much water is due to hydrogels being super absorbent polymers (SAPs). SAPs can swell and hold large amounts of water while maintaining their flexible structure due to their physical and chemical properties. Since hydrogels have such unique properties, there are many applications of hydrogels including but not limited to: contact lenses, tissue engineering, wound dressing, drug delivery, cat litter, and agricultural fertilizers. Hydrogels can be made from a variety of polymers which can be natural or synthetic. This study focuses on the use of the natural material- gelatin. Gelatin is a type of protein that is produced by the partial hydrolysis of collagen, which in a protein made up of amino acids. Simplified, gelatin is made from the breakdown of collagen using a reaction containing water [3]. Gelatin is a non-toxic, biodegradable, and natural polymer that is commonly used to form hydrogels [4], e.g., making Jello. One unique property of gelatin is that gel formation is reversible when reheated [5]. Gelatin is favorable for creating hydrogels due to its easy accessibility and low cost, but gelatin alone can sometimes have poor mechanical and chemical stability [4]. This can be improved in the presence of a Hofmeister ion, which can strengthen the gelatin-based hydrogel and improve its properties. With a stronger hydrogel, both the viscosity and modulus are expected to increase due to the presence of a kosmotropic ion [7]. This will strengthen the hydrogel overall, allowing it to be more useful in various applications.

Hofmeister's Ions

Since gelatin-based hydrogels alone are often weak and brittle, Hofmeister ions can be used to strengthen the gels. Hydrogels can be formed in salt solutions to improve their mechanical properties. When the gel is forming, it will soak up the ions in the solution. This occurs due to the processes of salting-out and salting-in. Salting-out occurs when strongly hydrated ions, most common in strongly hydrated anions, remove the water molecules from the protein, which will dehydrate the surface. This process will cause the gel to deswell. Salting-in is the process where the ionic strength of the salt solution increases the solubility of the gelatin, which results in the gel to swell in the solution. Salting-in is most common in weakly hydrated anions [6]. Hofmeister's ions are both cations and anions and are classified in series in the order based on their strength. The strongest cations and anions will best stabilize the protein [7]. It is important to note that the series will vary slightly depending on the protein that the ion is interacting with, but the series has been consistent across many studies.

$$NH_4^{\ +} > Cs^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ba^{2+}$$

**Figure 1:** Hofmeister series of cations [8].

$$SO_4^{2-} > HPO_4^{2-} > F^- > CH_3COO^- > Cl^- > Br^- > NO_3^- > I^- > ClO_4^- > SCN^-$$

**Figure 2:** Hofmeister series of anions [9].

## Experimental Methods

<u>Hofmeister Ions Solution Preparation</u>

All aspects of this study included the use of Hofmeister ion salt solutions. This study looked at six different salt solutions: sodium chloride, sodium sulfate, sodium carbonate, sodium thiocyanate, calcium chloride and ammonium chloride. Basic information about each salt used can be found in **Table 1** below. The salt solutions were created based on whatever concentration was necessary for the given experimental run. For a 2 M solution, 100 mL (0.1 L) of DI water was used and multiplied by the concentration of 2 M (2 mol/L) to determine the moles of salt needed. The amount in moles was then multiplied by the molar mass of the salt to determine the amount of salts in grams. The salt was carefully measured to the thousandths of a gram to ensure accuracy. The salt was added to a 100 mL volumetric flask and DI water was added. The flask was shook until all the salt was dissolved. These solutions were stored in a sealed container to be used in future studied. Additionally, the concentration could be lowered by adding the appropriate amount of solution and DI water.

**Table 1**: Information for all the Hofmeister ions of interest, including NaCl, $Na_2SO_4$, $Na_2CO_3$, NaSCN, $CaCl_2$, $NH_4Cl$, used in the study. These ions were used as salt solutions of varying molarity from 0.5 to 4 M to be used in swelling tests with bovine based gelatin.

| Salt | Molecular Formula | Molecular Weight (g/mol) | Density (g/cm3) | Solubility in water at RT (g/mL) |
|---|---|---|---|---|
| Sodium Chloride | NaCl | 58.44 | 2.16 | 0.384 |
| Sodium Sulfate | $Na_2SO_4$ | 142.04 | 2.66 | 0.497 |
| Sodium Carbonate | $Na_2CO_3$ | 105.99 | 2.54 | 0.341 |
| Sodium Thiocyanate | NaSCN | 81.072 | 1.74 | 1.39 |
| Calcium Chloride | $CaCl_2$ | 110.98 | 2.15 | 0.745 |
| Ammonium Chloride | $NH_4Cl$ | 53.491 | 1.53 | 0.458 |

<u>Solubility Study</u>

The first part of this project consisted of a solubility study to determine the amount of gelatin to use and the corresponding concentration of Hofmeister ion salt solution. This was vital to determine if the gelatin would dissolve in the solution in the future studies. In Dr. Newby's lab a prior study was conducted which examined the swelling of hydrogels in the Hofmeister ion solutions. This study looked at the six Hofmeister ion salt solutions previously mentioned: sodium chloride, sodium sulfate, sodium carbonate, sodium thiocyanate, calcium chloride and ammonium chloride. All of these were tested using 15 weight percent gel in each solution. The salt solutions varied from 1.136 – 2.919 M, depending on the solubility of the gelatin in that specific solution. No further concentrations were examined, so it was unknown if the gel is soluble at higher concentration. Also, the amount of gelatin was not varied, so further studies must be done to further understand the conditions in which gelatin is soluble.

Based on the previous study in Dr. Newby's lab, a concentration of 2 M salt solution was used as the baseline for starting the solubility study. Different amounts of gelatin pellets, first only 0.05 and 0.1 grams, were then placed in 1 mL of solution and heated at 37 °C to determine if the gelatin is soluble in the solution when heated. The gelatin/solution was then placed into a refrigerator. The next day, the solubility at 4 °C was recorded. The gelatin/solution was then left out in the lab so that the solubility at room temperature could be recorded. The state of the gelatin (soluble, gel, or not dissolved) was then recorded. When the salt was undissolved or it gelled up, it was considered insoluble. If the gelatin was able to dissolve easily, an increased gelatin concentration, increased solution concentration or a combination of both was tested to obtain more data. On the other hand, if the gelatin is insoluble, the gelatin or salt solution concentration was decreased and retested. The solubility data acted as a guide in determining which concentration of solutions could potentially form the strongest hydrogels.

Swelling Study

Most of the results from this project come from swelling tests. The first step in the swelling study was creating the gelatin hydrogels. To keep the conditions consistent, 10 wt% gelatin was used for all tests. The gelatin was made by weighing out 10 grams of the gelatin from bovine and porcine and added to a volumetric flask with 100 mL of DI water. The gelatin was dissolved by placing the flask on a hot plate and stirrer. Once the gelatin was completely dissolved, 1 gram of solution was deposited in each well of a 24 well-plate and cooled to room temperature to form gels. The gels were typically prepared 1 day before the swelling test took place.

When it was time to complete the swelling tests, the gel was removed and weighed for its initial mass. The gel was then placed in a second well-plate with each well containing a larger volume (either a 6 or 12 well-plate). If the gel was predicted to swell based on the salt solution it would soak in, it was placed in the 6 well-plate. If deswelling was predicted, the 12 well-plate was used. It became easier to predict if the gel would swell or deswell as testing continued as well as literature data of previous studies using the same Hofmeister ion solutions. Salt solution was added to the top of each well, ensuring that the entire gel was covered, and the entire well-plate was covered with a lid. The same salt solution was tested using three different gels to determine an average. It was important to clearly label all wells, so that the results could be recorded accurately. The gels were soaked for 2 hours and then removed and padded completely dry using a paper towel. The mass of the gel was measured and recorded and then the gel was placed back into the same well. The gels continued to soak and were removed again after 24 hours. Again, the gels were dried completely and the mass was recorded. If the gel dissolved in the solution at all, the swelling tests were repeated using the salt solution at a lower concentration. If the gel did not dissolve, the process was repeated with a new hydrogel in a solution of a higher concentration.

Gelatin Release Study

When gelatin is soaked in the salt solution, it is possible that some of the gelatin is released into the solution. To analyze this, after swelling was complete the gel was removed and a small amount of solution was used to measure the amount of gelatin in the solution using a microplate spectrometer at room temperature. Solutions were tested in triplicate to determine an average and to minimize error. A blank sample of DI water was used as a comparison. The measurement wavelength used was 235 nm. The absorbance for each solution was recorded. From the absorbance data a calibration curve (concentration vs absorbance) was created, which was used later to determine the concentration (wt%) of gelatin present in the solution. A positive concentration of gel found in the solution indicated the gelatin dissolved and/or released out from the hydrogel into the soaking solution.

## Data and Results

Solubility Study

The data for the solubility study was a mix of quantitative and qualitative observations. One salt solution could dissolve easily at 2 M, so the concentration of solution and the amount of gel could be increased. Another solution could not dissolve at all, so lower amounts of gel and lower concentration of salt solution would be needed. Labeling and recording all results clearly was important to keep track of all the results for all six salt solutions. The data for the solubility study was a mix of quantitative data and qualitative observations. The solutions had to be visually observed to determine if it was soluble or insoluble. It was important to note if the solutions were a liquid and all gel was dissolved (soluble) or if gel formed or the gelatin was completely undissolved (insoluble). Some images of both situations can be seen below in **Figure 3**.

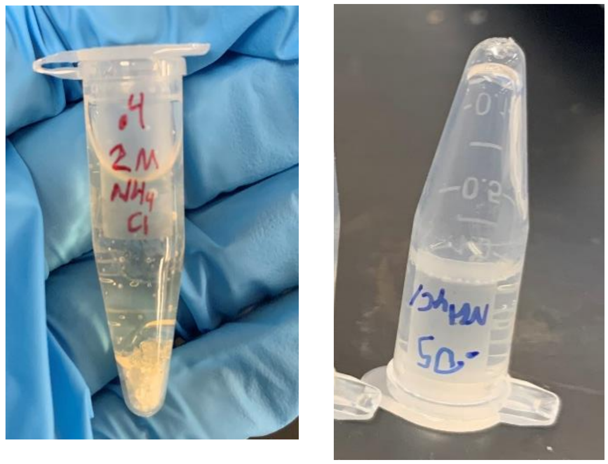


**Figure 3:** Example of gelatin insoluble in solution (left) and soluble in solution (right). The left picture was a test when 0.4 grams of gelatin was added to 2 M $NH_4Cl$. Some of the gelatin gelled up, while the other did not dissolve at all. The right picture is a test when 0.05 grams of gelatin was added to 2 M $NH_4Cl$. All the gelatin dissolved, and the solution was a liquid, indicating a soluble solution.

The gel % vs salt % graphs for each salt solution at all 3 temperatures (37 °C, room temperature (RT), and 4 °C) were created by simple calculations. The exact amount of gel added was recorded to the ten-thousandths of a gram. The weight of salt in the solution was calculated based on using 1 mL of solution (Equation 1) and then used with the amount of gel to calculate weight % gel (Equation 2) and weight % salt (Equation 3). A full summary of the results can be found in **Appendix A**.

**Equation 1:** $weight\ of\ salt\ \ (g) = molarity\ \left(\frac{mol}{L}\right) * MW\ of\ salt\ \left(\frac{g}{mol}\right) * 0.001(L)$

**Equation 2:** $weight\ \%\ gel = \frac{grams\ of\ gelatin}{(grams\ of\ gelatin + grams\ of\ solution)}$

**Equation 3:** $weight\ \%\ salt = \frac{grams\ of\ salt}{(grams\ of\ gelatin + grams\ of\ solution\ )}$

Once the weight % of gel and salt was calculated, all the results could be plotted. This was done for each of the six salt solutions with a different graph for each temperature (37 °C, room temperature (RT), and 4 °C). A solid point indicates the gelatin was soluble in the solution, while a hollow point indicates the gelatin was insoluble. The results can be seen in **Figures 4-9.**

**Figure 4** shows the results for NaCl. The gelatin for NaCl at the gel % and salt % tested were only soluble at 37 °C and insoluble at all other temperatures. For NaSCN in **Figure 5**, the hydrogels were soluble at all gel % and salt % tested at each temperature. No other concentrations were tested because it was assumed that similar results would be seen. The results for $Na_2SO_4$ can be seen in **Figure 6**. The hydrogels are soluble at 0.1532 and 0.2619 gel % at 4 °C and room temperature and soluble at 0.1532, 0.2619, 0.4170, and 0.6042 gel % at 37 °C . It is important to note that it was odd and unexpected that the hydrogel was soluble at 0.1532 and 0.2619 gel % for 4 °C and room temperature. This was thought to be a mistake, but the samples were retested twice and the results remained the same. It is recommended to retest at these values to see if the same trend remains. **Figure 7** shows all solubility results for $Na_2CO_3$. The gels were not soluble in the 2 M sodium carbonate solution. The hydrogels were insoluble at all gel % at 4 °C, and insoluble at 0.1942 gel % for both room temperature and 37 °C. $CaCl_2$ results can be found in **Figure 8**. The hydrogels were only soluble at all temperatures for a gel % of 0.1850 and 0.3165. At higher gel %, the gelatin becomes insoluble. Finally, results for $NH_4Cl$ can be found in **Figure 9**. The hydrogels were insoluble at all gel % for 4 C, insoluble at 0.6552 and 0.7893 gel % at room temperature, and insoluble at 0.7893 gel % at 37 °C. The gelatin was soluble at all other conditions tested at room temperature and 37 °C.

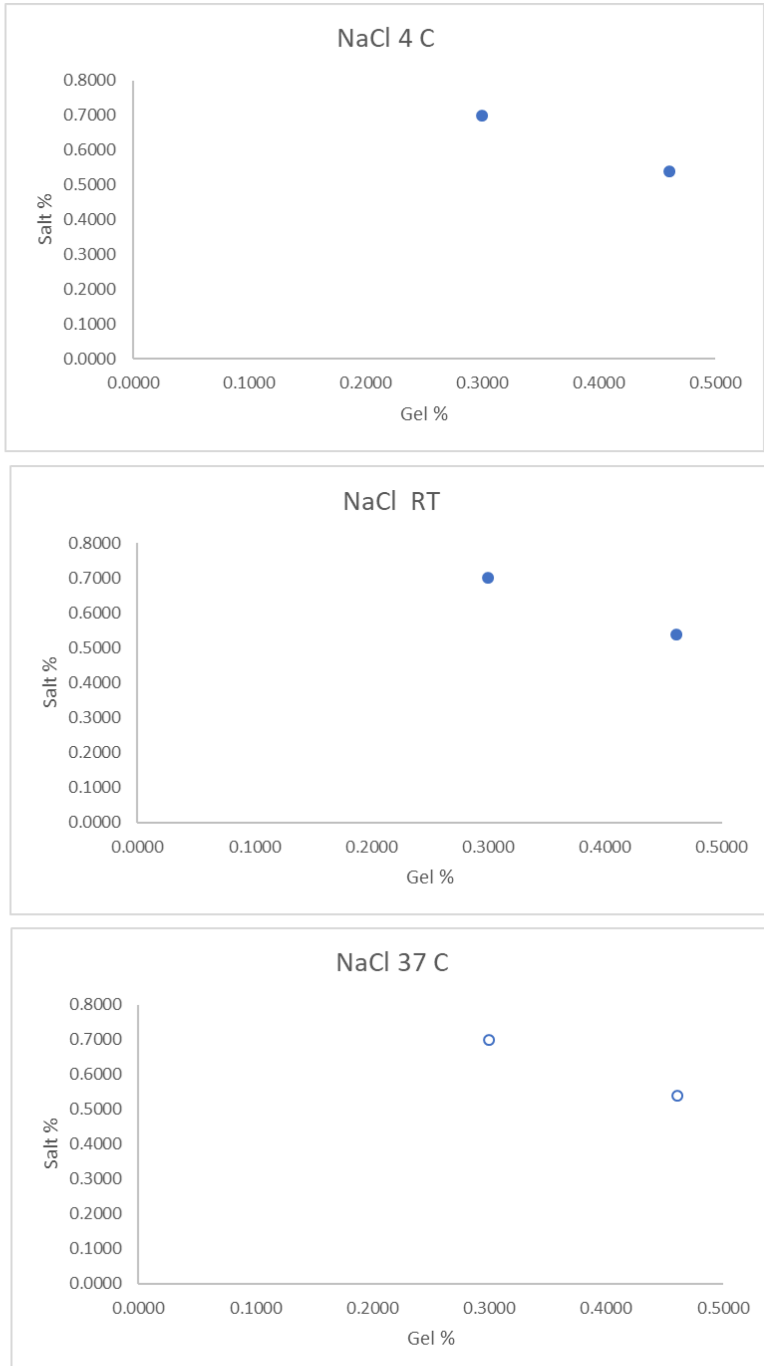


**Figure 4**: Solubility (open symbols – soluble, filled symbols – insoluble) of hydrogel in 2 M solution of NaCl at 4°C (top), room temperature (middle), and 37°C (bottom). Solubility is plotted as salt wt.% vs. gel wt.%.

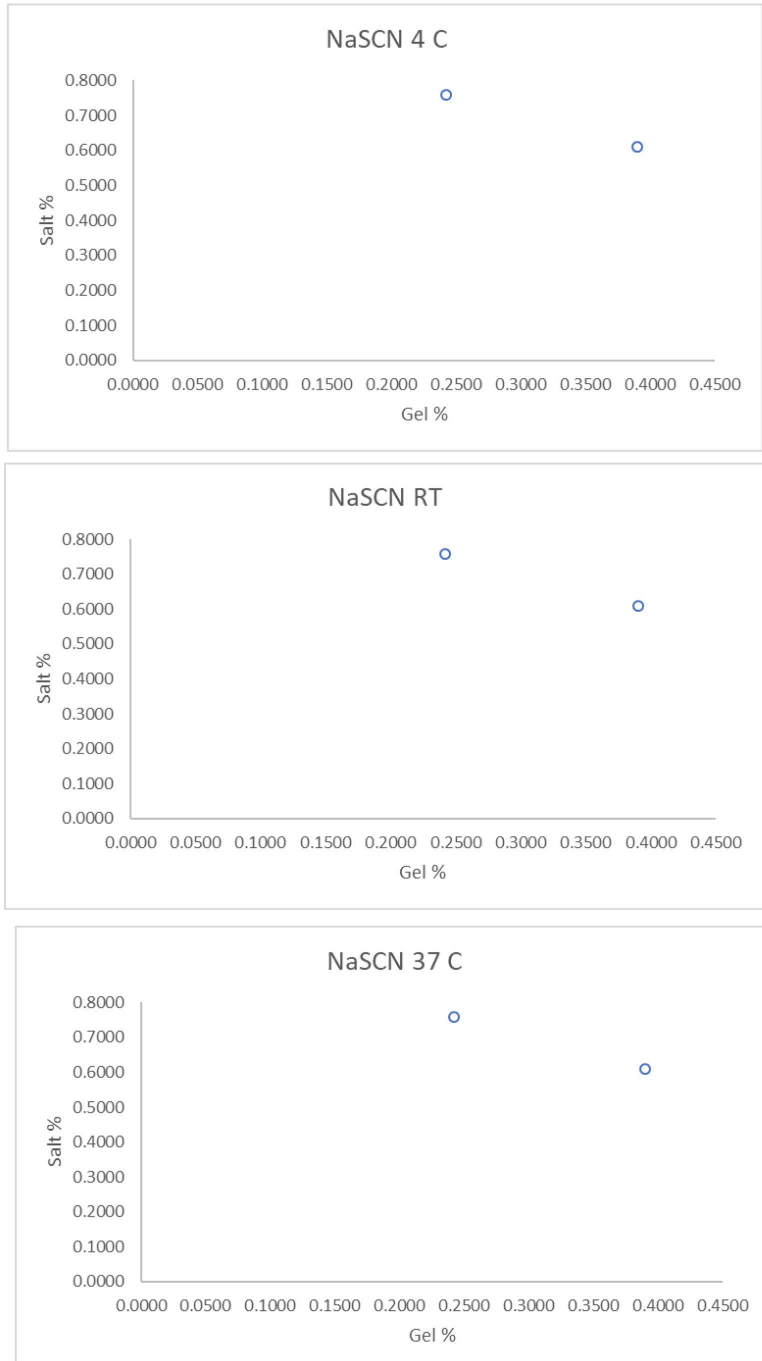


**Figure 5**: Solubility (open symbols – soluble) of hydrogel in 2M solutions of NaSCN at 4°C (top), room temperature (middle), and 37°C (bottom). Solubility is plotted as salt wt.% vs. gel wt.%.

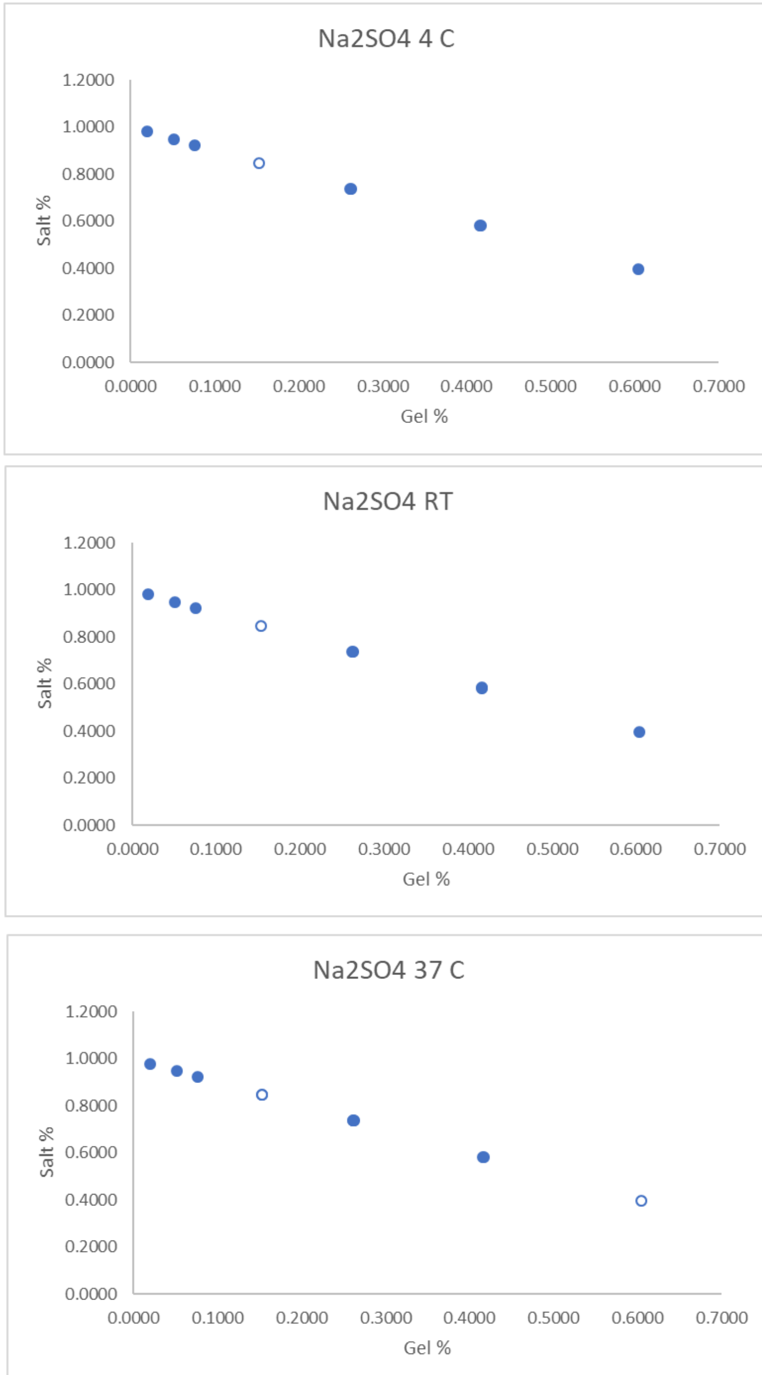


**Figure 6**: Solubility (open symbols – soluble, filled symbols – insoluble) of hydrogel in 0.5 M, 1 M, and 2 M solutions of $Na_2SO_4$ at 4 °C (top), room temperature (middle), and 37 °C (bottom). Solubility is plotted as salt wt.% vs. gel wt.%.


**Figure 7**: Solubility (open symbols – soluble, filled symbols – insoluble) of hydrogel in 1 M and 2 M solutions of $Na_2CO_3$ at 4°C (top), room temperature (middle), and 37°C (bottom). Solubility is plotted as salt wt.% vs. gel wt.%.

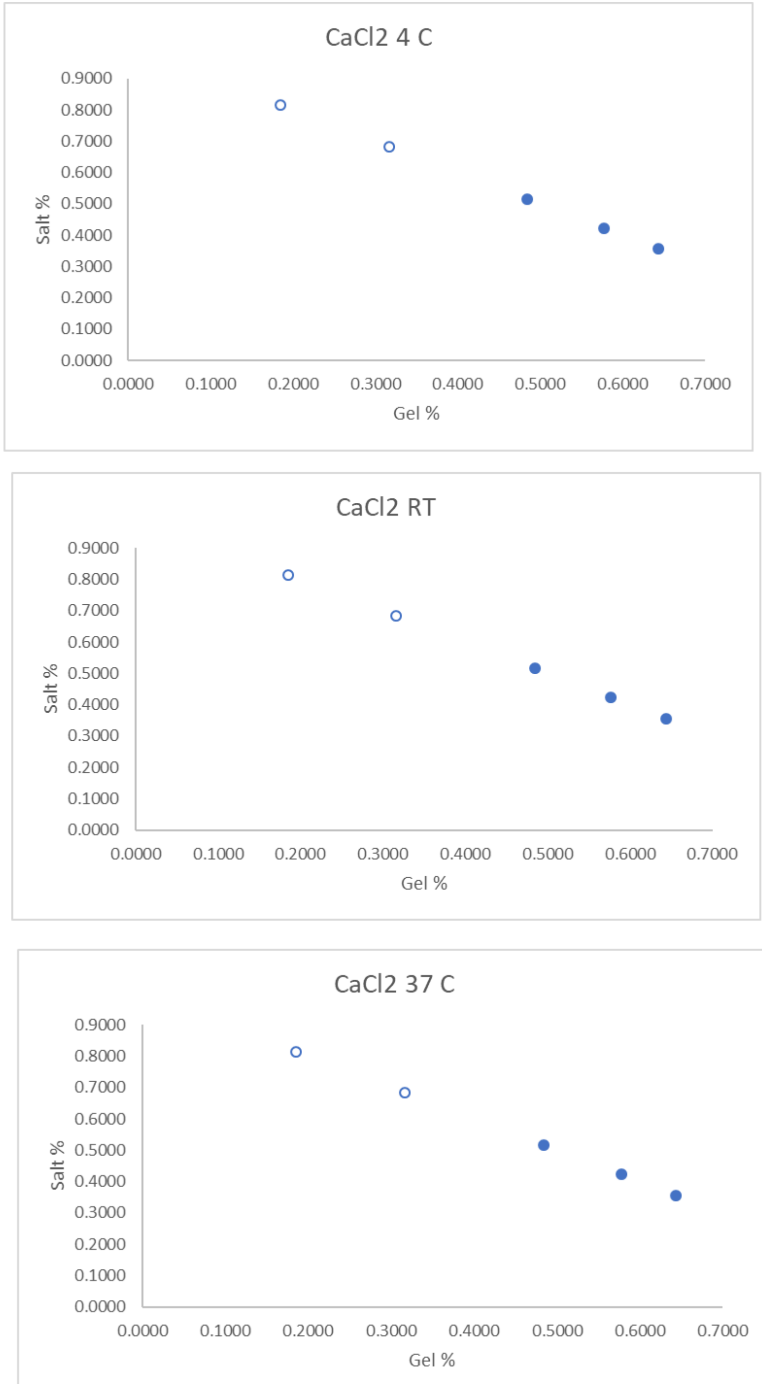


**Figure 8**: Solubility (open symbols – soluble, filled symbols – insoluble) of hydrogel in 2 M solution of $CaCl_2$ at 4 °C (top), room temperature (middle), and 37 °C (bottom). Solubility is plotted as salt wt.% vs. gel wt.%.

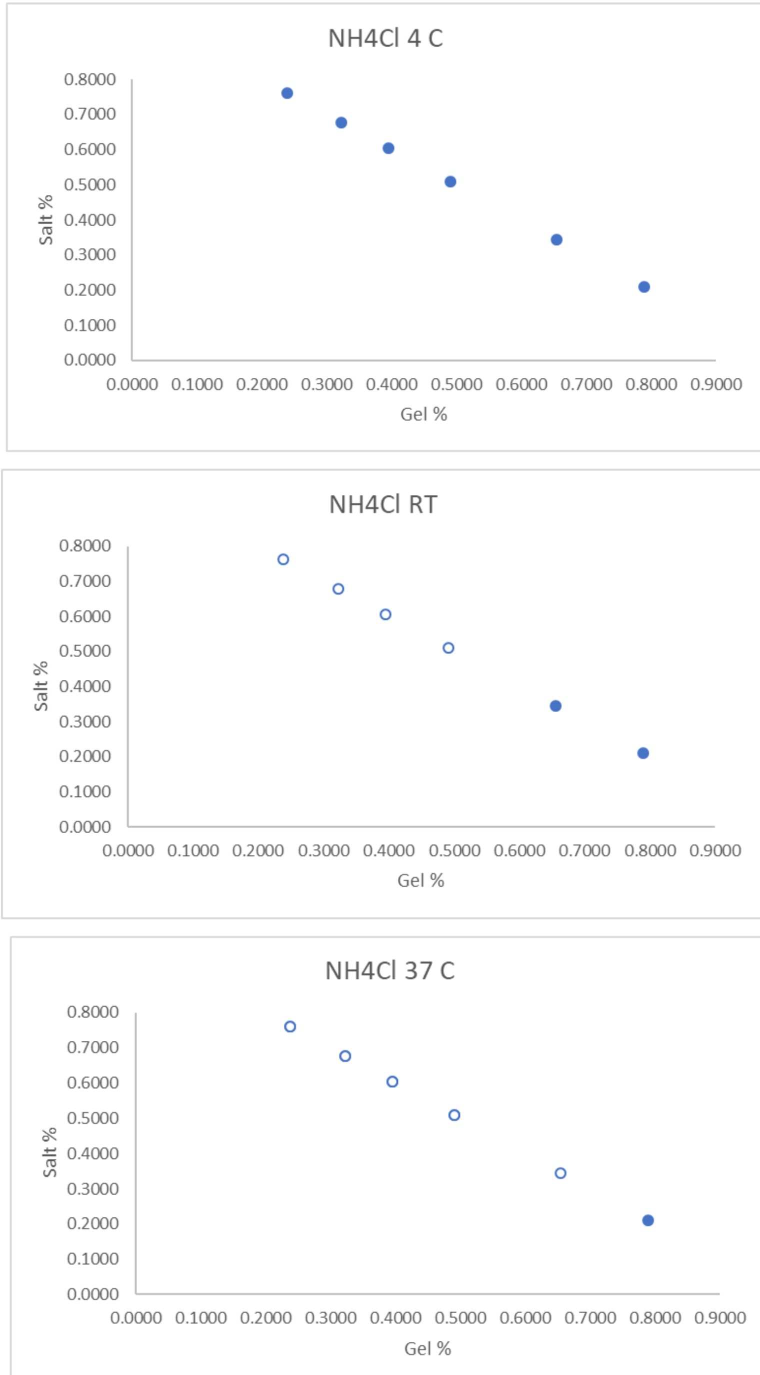


**Figure 9**: Solubility (open symbols – soluble, filled symbols – insoluble) of hydrogel in 2 M and 3 M solutions of $NH_4Cl$ at 4 °C (top), room temperature (middle), and 37 °C (bottom). Solubility is plotted as salt wt.% vs. gel wt.%.

Swelling Study

Various swelling tests were completed for sodium chloride, sodium sulfate, sodium carbonate, ammonium chloride and DI water at different concentrations. No swelling tests were completed for sodium thiocyanate or calcium chloride. This decision to not pursue swell tests was due to the solubility of 2 M sodium thiocyanate at all temperature. This result indicated that the gel would most likely dissolve into the solution, even at higher concentrations. Calcium chloride was also not tested based on solubility tests. It is a weak cation, suggesting that limited deswelling/swelling would be observed, or the gelatin would dissolve. For all tests, the swelling or deswelling was analyzed by the mass ratio which was measured by the final gel weight (after 2 hours and then again after 24 hours) divided by the initial gel weight before soaking in the salt solution. When the gel was swollen, the mass ratio was greater than one. When deswelling occurred, the mass ratio was less than one. Three or four samples were tested for each given concentration to establish an average and determine any error. The solubility study results acted as an aid to determine what solution concentrations should be tested. Any concentration in which the gelatin was soluble in would not be tested because the gelatin would dissolve. Predictions were also made based on what type of ion was being tested. For example, ammonium chloride is a kosmotropic cation, which predicts deswelling will occur. This prediction was based on the process of salting-out, which means the salt will take water away from gel. Results from the swelling tests for the four salt solutions as well as DI water can be found in **Figures 10-14**.

For all the swelling results, mass ratios above 1 indicates that the hydrogel swelled and mass ratios below 1 indicates that the hydrogel deswelled. The results for NaCl can be found in **Figure 10**. For run 1, swelling was only completed for 2 hours since testing for 24 hours was not decided until after that run was completed. Swelling occurred for both runs at both 2 hours and 24 hours. **Figure 11** shows swelling results for gelatin soaked in $Na_2SO_4$ solutions. When soaked in concentrations of 1 M and greater, the gelatin deswelled, while concentrations below 1 M showed the gelatin swelling. The 1 M test was only able to be measured after 24 hours and the 2 M was only able to be measured after 2 hours due to scheduling limitations. Similar results to the $Na_2SO_4$ soaked hydrogels were found when soaking in $Na_2CO_3$ found in **Figure 12**. Concentrations 1 M and greater showed deswelling, while concentrations 0.75 M and below showed the gelatin swelling. The 1 M test was only able to be measured after 24 hours and the 2

M was only able to be test after 2 hours due to scheduling limitations. Results for $NH_4Cl$ can be found in **Figure 13**. The gelatin swelled in both 2 M and 3 M solution. Swelling tests were only completed for 2 hours because any time greater would cause the gel to dissolve into the solution, making it impossible to get accurate measurements. The last swelling results can be found in **Figure 14**, which shows the results when using DI water. As predicted, the gels experience a lot of swelling to the hydrogel properties. For run 1, swelling was only completed for 2 hours. After that run, it was decided to begin testing again at 24 hours.

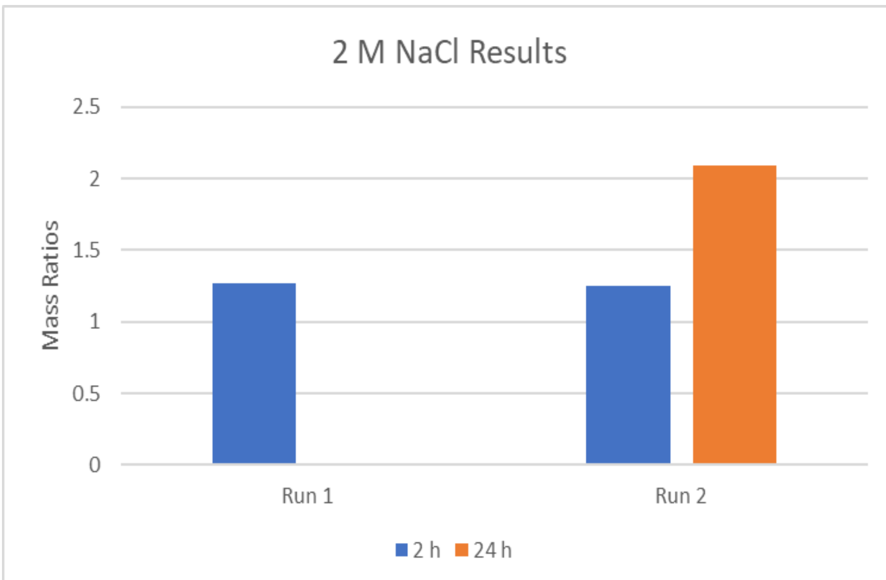


**Figure 10**: Swelling results for 2 M sodium chloride (NaCl) at 2 hours and 24 hours. Mass ratios above 1 indicates that the hydrogel swelled while mass ratios below 1 indicates that the hydrogel deswelled.

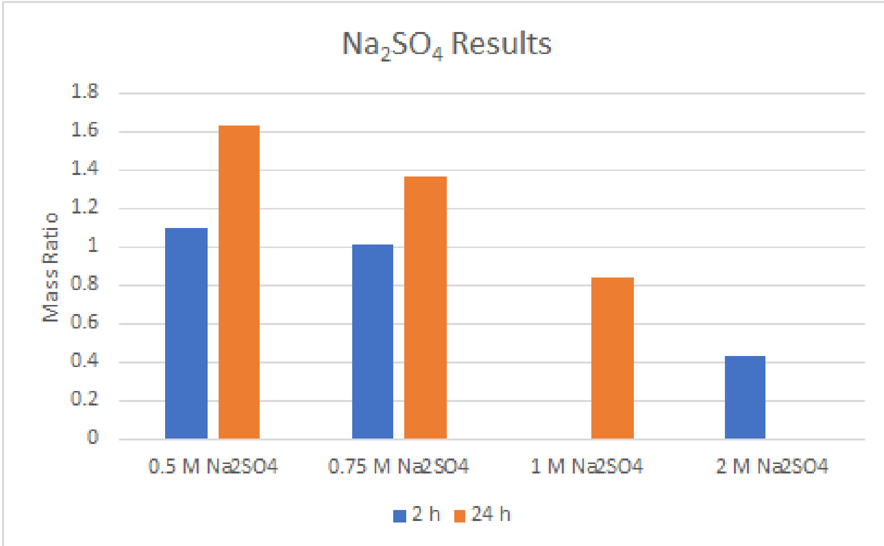


**Figure 11**: Swelling results for sodium sulfate ($Na_2SO_4$) at concentrations from 0.5 M to 2 M at 2 hours and 24 hours.

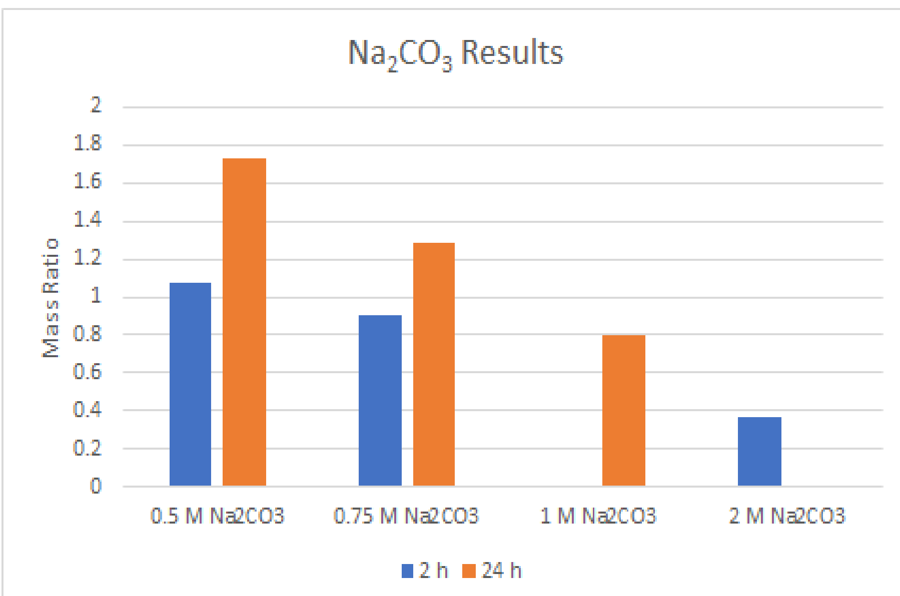


**Figure 12**: Swelling results for sodium carbonate ($Na_2CO_3$) at concentrations from 0.5 M to 2 M at 2 hours and 24 hours.

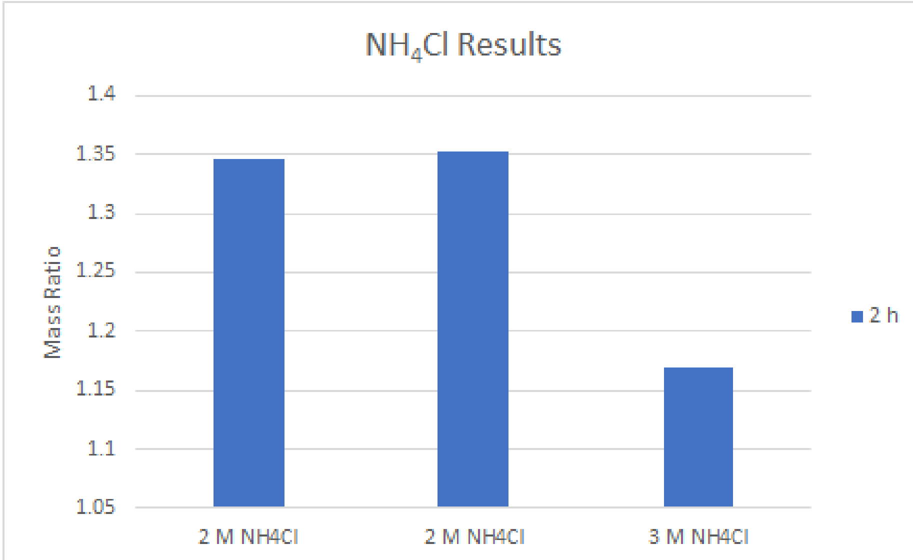


**Figure 13**: Swelling results for ammonium chloride ($NH_4Cl$) at concentrations at 2 M and 3 M at 2 hours.

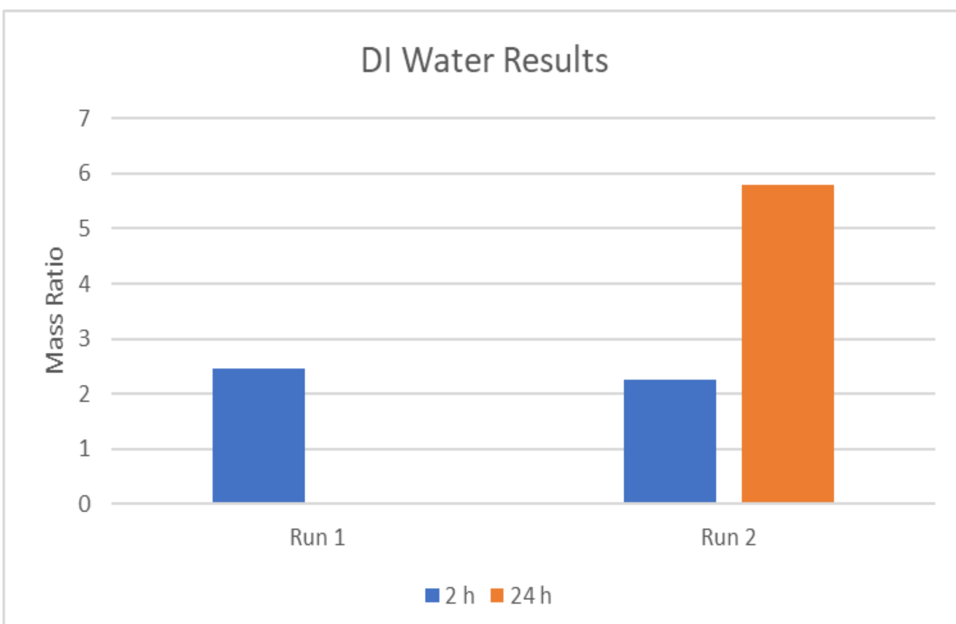


**Figure 14**: Swelling results for DI Water at 2 hours and 24 hours.

Gelatin Release Study

**Table 2:** Results from the absorbance readings using a spectrometer of different salt solutions after swelling tests. The average absorbance was based on the average of 3 measurements with standard error for each sample. The concentration (wt. %) of gelatin in the solution was calculated using average absorbance and the equation from the calibration curve, with standard error presented for each. This concentration is the amount of gelatin that was released into the solution or the gel dissolved. 4 M $NH_4Cl$ showed the most gel released (average of 0.233 wt%) due to the gelatin dissolving into the solution as seen during the swell test results.

| | Sample | Average Absorbance | Concentration (wt %) |
|---|---|---|---|
| **1 M Na2SO4** | 1 | 0.174 ± 0.015 | -0.045 ± 0.012 |
| | 2 | 0.164 ± 0.017 | -0.053 ± 0.014 |
| | 3 | 0.173 ± 0.014 | -0.046 ± 0.011 |
| **1 M Na2CO3** | 1 | 0.264 ± 0.032 | 0.029 ± 0.026 |
| | 2 | 0.22 ± 0.016 | -0.007 ± 0.013 |
| | 3 | 0.256 ± 0.006 | 0.022 ± 0.005 |
| **DI Water** | 1 | 0.189 ± 0.02 | -0.033 ± 0.016 |
| | 2 | 0.245 ± 0.048 | 0.013 ± 0.039 |
| | 3 | 0.206 ± 0.049 | -0.018 ± 0.04 |
| **2 M NaCl** | 1 | 0.212 ± 0.022 | -0.013 ± 0.018 |
| | 2 | 0.213 ± 0.029 | -0.013 ± 0.023 |
| | 3 | 0.249 ± 0.024 | 0.016 ± 0.019 |
| **2 M Na2SO4** | 1 | 0.167 ± 0.021 | -0.05 ± 0.017 |
| | 2 | 0.168 ± 0.005 | -0.049 ± 0.004 |
| | 3 | 0.161 ± 0.004 | -0.055 ± 0.003 |
| | 4 | 0.15 ± 0.006 | -0.064 ± 0.005 |
| **0.5 M Na2CO3** | 1 | 0.234 ± 0.003 | 0.004 ± 0.002 |
| | 2 | 0.232 ± 0.024 | 0.003 ± 0.019 |
| | 3 | 0.218 ± 0.011 | -0.009 ± 0.009 |
| | 4 | 0.205 ± 0.012 | -0.019 ± 0.01 |
| **4 M NH4Cl** | 1 | 0.521 ± 0.029 | 0.238 ± 0.023 |
| | 2 | 0.502 ± 0.032 | 0.222 ± 0.026 |
| | 3 | 0.49 ± 0.036 | 0.213 ± 0.029 |
| | 4 | 0.546 ± 0.042 | 0.258 ± 0.034 |
| **0.5 M Na2SO4** | 1 | 0.195 ± 0.015 | -0.027 ± 0.012 |
| | 2 | 0.199 ± 0.012 | -0.024 ± 0.01 |
| | 3 | 0.178 ± 0.016 | -0.041 ± 0.013 |
| | 4 | 0.192 ± 0.015 | -0.03 ± 0.013 |

It was expected that as the gelatin was soaked in solution, some of the gelatin would dissolve or release into the solution. To measure gelatin dissolution, a calibration curve was created and can be seen in **Appendix B, Figure 15**. This calibration curve was then used to test various solution samples after gelatin had been soaked in it for 24 hours. That solution had its absorbance tested and used the calibration curve to determine the concentration (wt %) that was present after swelling tests were completed. The results for various samples can be seen in **Table 2**. The results in this table show what the tested absorbance was for each sample. This absorbance was then used to calculate the gelatin concentration that was present in the sample. A positive concentration indicates that there was gelatin present in the solution. If gelatin was present, then it dissolved into the solution while it was soaking. A more detailed table of **Table 2**, which includes error for all absorbances and concentrations can be found in **Appendix C (Figure C)**.

## Discussion

<u>Solubility Study</u>

The solubility study was a vital part of the project. The results from the solubility studies helped guide the swelling tests. Since sodium thiocyanate (NaSCN) is such a strong ion, gelatin was soluble at all salt % and gel %. Because of this, it was decided to not pursue the swelling tests using this salt solution. A very low concentration would need to be used and even then, the gelatin may have dissolved in the solution. Additionally, calcium chloride ($Ca_2Cl$) showed that gelatin was often soluble. It was the only other salt solution, besides NaSCN, in which gelatin dissolved at 4 °C. This indicated that swelling tests would be unsuccessful. With more time and resources, these trends could be further examined at different solution concentrations as well as different gelatin amounts.

There are some recommendations that could help improve this study. First, retesting of the sodium sulfate must be done. Gelatin solubility was scattered and inconsistent with any trend observed with the other salt solutions. Although no errors in sample preparation were identified, samples were completed by two different people throughout the study and selected retesting afforded the same results. It is suggested that one person sets up each sample to reduce any human error in the samples. Finally, a tertiary phase diagram should be completed for each salt solution. This would allow an easy visualization on how temperature, gel % and salt % impacts the solubility.

Swelling Study

As expected, swelling was most prominent when the gelatin was soaked in DI water. For both runs tested, the mass ratio was greater than 2 after 2 hours and greater than 5 after 24 hours. This was not a surprise as hydrogels are hydrophilic. Like DI water, swelling occurred for hydrogels soaked in 2 M sodium chloride (NaCl) solution. Swelling increased and the mass ratio was larger as time went on and the gelatin soaked more. It was expected that similar results would be seen at higher concentrations, so no additional tests were completed. When using ammonium chloride ($NH_4Cl$), the hydrogel also swelled. Concentrations of 1 M, 2 M and 3 M were tested and although swelling decreased at the highest concentration, it still showed similar results. At all concentrations the gelatin would swell after 2 hours, but it would begin to disintegrate into the solution. Because of this, no results were able to be obtained beyond 2 hours.

Since the goal of this study was to create strong, inexpensive hydrogels to be used in various biomedical applications, the most promising results came from gelatin that was able to deswell in solution. The deswelling causes the hydrogels to go from weak and brittle to strong and durable. Two of the salts tested, sodium sulfate ($Na_2SO_4$) and sodium carbonate ($Na_2CO_3$), showed results in which the gelatin deswelled. When the gelatin was soaked at molarities of 0.5 M and 0.75 M, swelling occurred at both 2 hours and increased when soaked for 24 hours. When the molarity of the solution was increased to 1 M and 2 M, the gelatin deswelled. This result was promising and showed that it is possible for the hydrogel to deswell in solution. Like sodium sulfate, sodium carbonate also shared the same trend. The hydrogel deswelled when soaked in 1 M and 2 M sodium carbonate solution.

After reviewing all results, sodium sulfate and sodium carbonate should be further investigated. The greatest deswelling, or shrinkage, by the hydrogel was observed when 2 M salt solution was tested. Higher concentrations should be tested to see if the deswelling could be optimized. Stronger hydrogels may be possible, with a small increase in cost. It should also be tested to see what happens when the hydrogels soak for greater than 24 hours. There may be a limit where no significant swelling/deswelling occurs, but that is unknown. This could be easily observed if the hydrogels remained soak in additional 6, 12, or 24-hour increments.

Gelatin Release Study

Based on **Table 2,** many concentrations of gelatin in solution seem to be negative, but very close to zero. This is predicted to be due to inherent instrument error and detection limitations of the spectrometer when the calibration curve was created and when absorbance of samples was measured. The machine was very sensitive, and any small error could cause inaccuracy. It is assumed that very small negative values, like -0.045 wt% for 1 M $Na_2SO_4$, represents no gelatin was released or dissolved into the solution. The only noticeable results come from 4 M $NH_4Cl$. The hydrogels for this swelling test was unable to be used in results as the gels were falling apart when taken out of the solution. There was no way to accurately weigh the swollen hydrogels. However, the solution in which the gels soaked in was evaluated. Of the four samples tested, the concentration of gel in solution ranged from 0.213 wt% to 0.258 wt%. This solution was the only salt solution tested that showed any indication that gel was released into the solution. This was expected as ammonium chloride was the only solution used in which the gelatin began to disintegrate during swelling tests. When the disintegration occurred, the gelatin was releasing into the solution.

To improve results and for future studies, more understanding should be given about the spectrometer. It was difficult to measure out such small samples for the plate used in the spectrometer. There is a chance there was some human error or slight cross contamination of samples. Results may be improved if repeated and there was better precision. The best option would to get new data for a new calibration curve. Since some concentrations of gelatin in solution came out as negative, the calibration curve equation might have been the issue. Also, a wavelength of 235 nm was chosen, but there may be a better option for this analysis. More optimization of the process could result in more accurate findings.

## Conclusion

Hydrogels are known to be weak and brittle, due to the amounts of water that they hold. Gelatin-based hydrogels specifically are inexpensive and easily accessible, but often have poor physical properties. The goal of my project was to strengthen gelatin-based hydrogels and improve their mechanical properties by forming these hydrogels in different aqueous solutions containing kosmotropic Hofmeister's ions. Prior research showed that these ions were able to take water away from gelatin-based gels, which forms a tougher hydrogel. The success of this project was measured by studying the solubility and swelling effects that different Hofmeister ions have on these gelatin-based hydrogels. Solubility studies provided correlation on how temperature, salt solution concentration, and gelatin concentration correlate to gel solubility. The solubility data was used further for the swelling/de-swelling studies. Through various swelling/de-swelling tests, an optimal salt solution containing a Hofmeister ion which toughen the hydrogel was found. Both sodium sulfate and sodium carbonate with molarities greater than 1 M can strengthen the hydrogel, which then improves the mechanical properties of the hydrogel.

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**Appendix A: Solubility Raw Data**

**Table A:** All the solubility results based on the solubility study. This data was used to plot the salt % vs gel % graphs at each temperature.

| Solution | Molarity | Grams of Gel | 4°C | RT | 37°C | Weight of Soln | Total Grams | Weight % Gel | Weight % Salt |
|---|---|---|---|---|---|---|---|---|---|
| NaCl | 2 | 0.05 | Insoluble | Insoluble | Soluble | 0.1169 | 0.1669 | 0.2996 | 0.7004 |
| NaCl | 2 | 0.1 | Insoluble | Insoluble | Soluble | 0.1169 | 0.2169 | 0.4611 | 0.5389 |
| $CaCl_2$ | 2 | 0.0504 | Soluble | Soluble | Soluble | 0.2220 | 0.2724 | 0.1850 | 0.8150 |
| $CaCl_2$ | 2 | 0.1028 | Soluble | Soluble | Soluble | 0.2220 | 0.3248 | 0.3165 | 0.6835 |
| $CaCl_2$ | 2 | 0.2086 | Insoluble | Insoluble | Insoluble | 0.2220 | 0.4306 | 0.4845 | 0.5155 |
| $CaCl_2$ | 2 | 0.3031 | Insoluble | Insoluble | Insoluble | 0.2220 | 0.5251 | 0.5773 | 0.4227 |
| $CaCl_2$ | 2 | 0.4009 | Insoluble | Insoluble | Insoluble | 0.2220 | 0.6229 | 0.6436 | 0.3564 |
| $NH_4Cl$ | 2 | 0.051 | Insoluble | Soluble | Soluble | 0.1070 | 0.1580 | 0.3228 | 0.6772 |
| $NH_4Cl$ | 2 | 0.1031 | Insoluble | Soluble | Soluble | 0.1070 | 0.2101 | 0.4908 | 0.5092 |
| $NH_4Cl$ | 2 | 0.2033 | Insoluble | Insoluble | Soluble | 0.1070 | 0.3103 | 0.6552 | 0.3448 |
| $NH_4Cl$ | 2 | 0.4008 | Insoluble | Insoluble | Insoluble | 0.1070 | 0.5078 | 0.7893 | 0.2107 |
| $NH_4Cl$ | 3 | 0.0503 | Insoluble | Soluble | Soluble | 0.1605 | 0.2108 | 0.2386 | 0.7614 |
| $NH_4Cl$ | 3 | 0.1049 | Insoluble | Soluble | Soluble | 0.1605 | 0.2654 | 0.3953 | 0.6047 |
| $Na_2SO_4$ | 2 | 0.0514 | Soluble | Soluble | Soluble | 0.2841 | 0.3355 | 0.1532 | 0.8468 |
| $Na_2SO_4$ | 2 | 0.1008 | Soluble | Soluble | Soluble | 0.2841 | 0.3849 | 0.2619 | 0.7381 |
| $Na_2SO_4$ | 2 | 0.0234 | Insoluble | Insoluble | Insoluble | 0.2841 | 0.3075 | 0.0761 | 0.9239 |
| $Na_2SO_4$ | 2 | 0.0153 | Insoluble | Insoluble | Insoluble | 0.2841 | 0.2994 | 0.0511 | 0.9489 |
| $Na_2SO_4$ | 2 | 0.0056 | Insoluble | Insoluble | Insoluble | 0.2841 | 0.2897 | 0.0193 | 0.9807 |
| $Na_2SO_4$ | 1 | 0.1013 | Insoluble | Insoluble | Insoluble | 0.1420 | 0.2433 | 0.4163 | 0.5837 |
| $Na_2SO_4$ | 1 | 0.0507 | Insoluble | Insoluble | Insoluble | 0.1420 | 0.1927 | 0.2630 | 0.7370 |
| $Na_2SO_4$ | 0.5 | 0.0508 | Insoluble | Insoluble | Soluble | 0.0710 | 0.1218 | 0.4170 | 0.5830 |
| $Na_2SO_4$ | 0.5 | 0.1084 | Insoluble | Insoluble | Soluble | 0.0710 | 0.1794 | 0.6042 | 0.3958 |
| NaSCN | 2 | 0.0517 | Soluble | Soluble | Soluble | 0.1621 | 0.2138 | 0.2418 | 0.7582 |
| NaSCN | 2 | 0.1037 | Soluble | Soluble | Soluble | 0.1621 | 0.2658 | 0.3901 | 0.6099 |
| $Na_2CO_3$ | 2 | 0.0511 | Insoluble | Insoluble | Insoluble | 0.2120 | 0.2631 | 0.1942 | 0.8058 |
| $Na_2CO_3$ | 1 | 0.0522 | Insoluble | Soluble | Soluble | 0.1060 | 0.1582 | 0.3300 | 0.6700 |
| $Na_2CO_3$ | 1 | 0.1075 | Insoluble | Soluble | Soluble | 0.1060 | 0.2135 | 0.5035 | 0.4965 |

**Appendix B: Gelatin Release Calibration Curve**

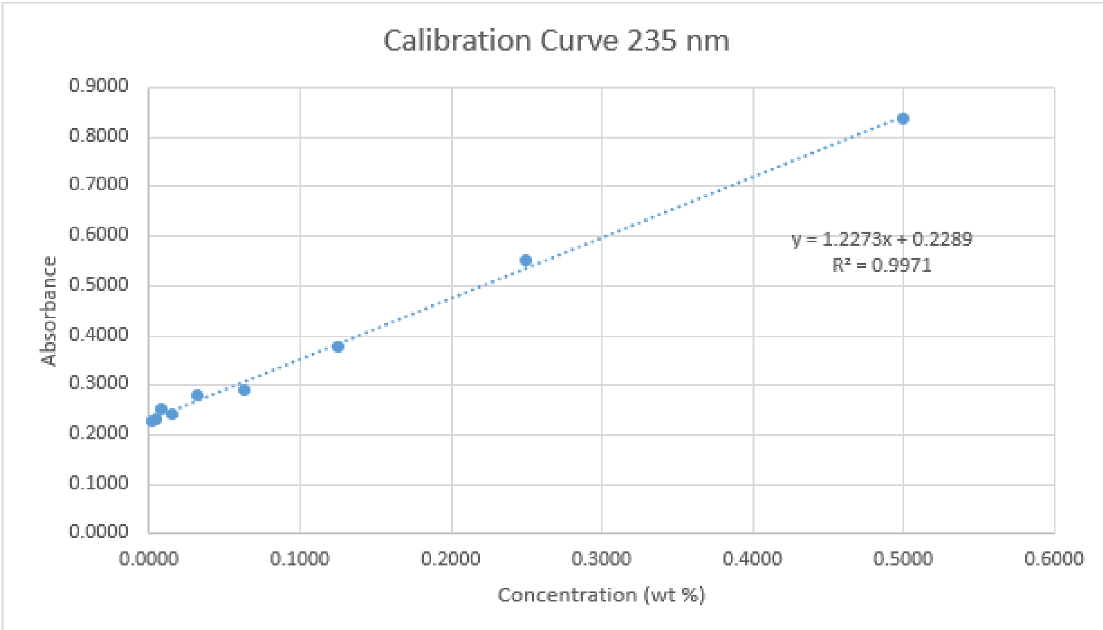


**Figure B**: Calibration Curve for the data from 9-26-2019 using a measurement wavelength of 235 nm. This calibration curve was created by a series of gelatin solution in DI water. Samples ranged from 1 wt.% gelatin to 0.0078 wt.%. The concentration of gelatin was cut in half for each new sample. This data was then used with the absorbance data for the gelatin release study to determine the concentration (wt%) of gel that was dissolved into the solution after swell tests were complete.

## Appendix C: Gelatin Release Detailed Table

**Table C:** All the results based on the gelatin release study. This data was used to find the average absorbance and concentration of each sample tested, including standard error. This is a more detailed version of **Table 2**.

| | | Absorbance | | | | | Concentration | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Sample #** | **Average** | **StDev** | **Low** | **High** | **Final** | **Average** | **Low** | **High** | **Variance** | **Final** |
| **1 M Na2SO4** | 1 | 0.174 | 0.015 | 0.16 | 0.189 | 0.174 ± 0.015 | -0.045 | -0.056 | -0.033 | 0.012 | -0.045 ± 0.012 |
| | 2 | 0.164 | 0.017 | 0.147 | 0.182 | 0.164 ± 0.017 | -0.053 | -0.067 | -0.038 | 0.014 | -0.053 ± 0.014 |
| | 3 | 0.173 | 0.014 | 0.159 | 0.186 | 0.173 ± 0.014 | -0.046 | -0.057 | -0.035 | 0.011 | -0.046 ± 0.011 |
| **1 M Na2CO3** | 1 | 0.264 | 0.032 | 0.232 | 0.297 | 0.264 ± 0.032 | 0.029 | 0.002 | 0.055 | 0.026 | 0.029 ± 0.026 |
| | 2 | 0.22 | 0.016 | 0.205 | 0.236 | 0.22 ± 0.016 | -0.007 | -0.02 | 0.006 | 0.013 | -0.007 ± 0.013 |
| | 3 | 0.256 | 0.006 | 0.25 | 0.262 | 0.256 ± 0.006 | 0.022 | 0.017 | 0.027 | 0.005 | 0.022 ± 0.005 |
| **DI Water** | 1 | 0.189 | 0.02 | 0.169 | 0.208 | 0.189 ± 0.02 | -0.033 | -0.049 | -0.017 | 0.016 | -0.033 ± 0.016 |
| | 2 | 0.245 | 0.048 | 0.197 | 0.293 | 0.245 ± 0.048 | 0.013 | -0.026 | 0.052 | 0.039 | 0.013 ± 0.039 |
| | 3 | 0.206 | 0.049 | 0.158 | 0.255 | 0.206 ± 0.049 | -0.018 | -0.058 | 0.021 | 0.04 | -0.018 ± 0.04 |
| **2 M NaCl** | 1 | 0.212 | 0.022 | 0.19 | 0.234 | 0.212 ± 0.022 | -0.013 | -0.031 | 0.004 | 0.018 | -0.013 ± 0.018 |
| | 2 | 0.213 | 0.029 | 0.184 | 0.242 | 0.213 ± 0.029 | -0.013 | -0.036 | 0.01 | 0.023 | -0.013 ± 0.023 |
| | 3 | 0.249 | 0.024 | 0.225 | 0.273 | 0.249 ± 0.024 | 0.016 | -0.003 | 0.036 | 0.019 | 0.016 ± 0.019 |
| **2 M Na2SO4** | 1 | 0.167 | 0.021 | 0.146 | 0.189 | 0.167 ± 0.021 | -0.05 | -0.068 | -0.033 | 0.017 | -0.05 ± 0.017 |
| | 2 | 0.168 | 0.005 | 0.163 | 0.173 | 0.168 ± 0.005 | -0.049 | -0.054 | -0.045 | 0.004 | -0.049 ± 0.004 |
| | 3 | 0.161 | 0.004 | 0.158 | 0.165 | 0.161 ± 0.004 | -0.055 | -0.058 | -0.052 | 0.003 | -0.055 ± 0.003 |
| | 4 | 0.15 | 0.006 | 0.144 | 0.157 | 0.15 ± 0.006 | -0.064 | -0.069 | -0.059 | 0.005 | -0.064 ± 0.005 |
| **0.5 M Na2CO3** | 1 | 0.234 | 0.003 | 0.231 | 0.236 | 0.234 ± 0.003 | 0.004 | 0.002 | 0.006 | 0.002 | 0.004 ± 0.002 |
| | 2 | 0.232 | 0.024 | 0.209 | 0.256 | 0.232 ± 0.024 | 0.003 | -0.016 | 0.022 | 0.019 | 0.003 ± 0.019 |
| | 3 | 0.218 | 0.011 | 0.207 | 0.228 | 0.218 ± 0.011 | -0.009 | -0.017 | 0 | 0.009 | -0.009 ± 0.009 |
| | 4 | 0.205 | 0.012 | 0.193 | 0.218 | 0.205 ± 0.012 | -0.019 | -0.029 | -0.009 | 0.01 | -0.019 ± 0.01 |
| **4 M NH4Cl** | 1 | 0.521 | 0.029 | 0.492 | 0.549 | 0.521 ± 0.029 | 0.238 | 0.214 | 0.261 | 0.023 | 0.238 ± 0.023 |
| | 2 | 0.502 | 0.032 | 0.47 | 0.533 | 0.502 ± 0.032 | 0.222 | 0.196 | 0.248 | 0.026 | 0.222 ± 0.026 |
| | 3 | 0.49 | 0.036 | 0.455 | 0.526 | 0.49 ± 0.036 | 0.213 | 0.184 | 0.242 | 0.029 | 0.213 ± 0.029 |
| | 4 | 0.546 | 0.042 | 0.504 | 0.588 | 0.546 ± 0.042 | 0.258 | 0.224 | 0.292 | 0.034 | 0.258 ± 0.034 |
| **0.5 M Na2SO4** | 1 | 0.195 | 0.015 | 0.18 | 0.21 | 0.195 ± 0.015 | -0.027 | -0.039 | -0.015 | 0.012 | -0.027 ± 0.012 |
| | 2 | 0.199 | 0.012 | 0.188 | 0.211 | 0.199 ± 0.012 | -0.024 | -0.034 | -0.015 | 0.01 | -0.024 ± 0.01 |
| | 3 | 0.178 | 0.016 | 0.162 | 0.194 | 0.178 ± 0.016 | -0.041 | -0.054 | -0.028 | 0.013 | -0.041 ± 0.013 |
| | 4 | 0.192 | 0.015 | 0.176 | 0.207 | 0.192 ± 0.015 | -0.03 | -0.043 | -0.018 | 0.013 | -0.03 ± 0.013 |